Express Mail No.: EL 501 639 779 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Kroczek

Application No.: To be assigned | Group Art Unit: To be assigned

Filed: Herewith | Examiner: To be assigned

For: METHODS FOR TREATMENT OF ASTHMATIC DISORDERS (as amended) | Attorney Docket No.: 7853-240

DECLARATION OF RICHARD KROCZEK UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, RICHARD KROCZEK do declare and state:

1. I am the inventor of the invention described and claimed in the above-identified patent application.

2. I presently hold the position of Professor of Molecular Immunology at the Robert Koch Institute, Berlin, Germany, the assignee of the above-identified patent application. My *curriculum vitae* is attached hereto as Exhibit 1.

3. I have read and am familiar with the instant application.

4. The invention claimed in the present application is directed to methods of treating asthmatic disorders comprising administering monoclonal antibodies directed against a human polypeptide, referred to in the present application as the "8F4 polypeptide." Since the original filing date of the specification of PCT Application PCT/DE98/02896, to which the present application claims priority, the 8F4 polypeptide has come to be referred to in the literature as "ICOS" (Inducible T cell Co-Stimulator). A polypeptide corresponding to an 8F4 polypeptide has also been referred to in the literature as "H4."

5. Described in paragraphs 6 to 11 below is a study by Gonzalo *et al.* (2001, "ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses," Nat. Immunol. 2(7):597-604, attached hereto as Exhibit 2)("Gonzalo"), of which I am a co-author, that demonstrates that administration of ICOS-inhibitory compounds, such an antibody that recognizes ICOS and an ICOS polypeptide, in an accepted experimental model of human asthma, result in abrogation of symptoms of the disease. Further described in paragraphs 12 to 14 below are experiments done by me or by others under my supervision that demonstrate that ICOS-expressing cells are associated with lung inflammation resulting in asthma in humans. These experiments corroborate the teaching provided in the present application by indicating that successful *in vivo* amelioration of asthma symptoms can be achieved by administration of antibodies that recognize the human ICOS polypeptide or the ICOS polypeptide itself.

INHIBITION OF ASTHMA SYMPTOMS BY ICOS-INHIBITORY MOLECULES IN A MOUSE MODEL OF ASTHMA

6. The study reported by Gonzalo provides evidence of the critical role of ICOS in the pathology of asthma. The experiments described by Gonzalo utilize an art-recognized mouse model for asthma, DO11.10 (Wills-Karp, 2000, Immunopharmacology 48:263-268, attached hereto as Exhibit 3). DO11.10 transgenic mice express a T cell receptor specific for the OVA antigen (amino acid residues 323-339 of chicken ovalbumin). Briefly, upon exposure to particular antigens, DO11.10 mice exhibit symptoms (e.g., lung mucosal inflammation) that are characteristic of asthma in humans. The severity of the symptoms in such treated mice was notably reduced by administration of ICOS inhibitory molecules to the mice.

7. More specifically, exposure of DO11.10 mice to the OVA antigen results in the production of a variety of inflammation-promoting cytokines in the bronchioalveolar fluid (BAL) of the mice. In contrast, as described in the Section at page 600 entitled "ICOS- and CD28-mediated cytokine regulation" and the accompanying Figure 7, administration of a monoclonal antibody that recognizes ICOS, 12A8, prior to exposure of the mice to the OVA antigen reduces the levels of inflammatory cytokines in the BAL of the mice relative to untreated DO11.10 mice. Similarly, administration of a soluble form of ICOS polypeptide, ICOS-Ig, prior to exposure of the mice to the OVA antigen reduces the levels of inflammatory cytokines in the BAL of the mice relative to untreated DO11.10 mice. Thus, administration of a monoclonal antibody that recognizes ICOS or an ICOS polypeptide reduces a hallmark of asthma.

8. Another hallmark of asthma in humans is accumulation of lymphocytes and eosinophils in the BAL upon exposure to antigenic stimulus. After persistent exposure to OVA, DO11.10 mice exhibit such accumulation. As described in the Section on page 599 entitled "Regulation of mucosal inflammation by ICOS" and Figure 5, the administration of 12A8 antibody DO11.10 mice before each exposure to OVA reduces lymphocyte and eosinophil accumulation by 50 and 70%, respectively. The authors of Gonzalo further note, also as shown in Figure 5, that comparable suppression of lymphocyte and eosinophil accumulation observed upon administering ICOS-Ig. Again, this represents a reduction in a hallmark of asthma.

9. In summary, the experimental results presented in Gonzalo demonstrate that administration of ICOS inhibitory compounds, such as anti-ICOS antibodies or ICOS proteins, can ameliorate symptoms of asthma.

ICOS IN HUMAN ASTHMA

10. The results presented hereinbelow demonstrate that ICOS is, indeed, involved in human lung inflammation such as that seen in asthma. This human data further evidences that administration of ICOS inhibitory compounds to humans will mirror the effects of administering such compounds to the art accepted model of human asthma, and accordingly, that ICOS inhibitory compounds will be useful in treating human asthmatic disorders.

11. Airway hyperresponsiveness and pulmonary inflammation are the two central hallmarks of human allergic asthma. The inflammatory process is initiated and maintained by T cells. The crucial role of certain co-stimulatory pathways in allergic asthma has been demonstrated in mice and several studies in the human (Exhibits 4-5).

12. To assess the participation of the ICOS molecule in the pathogenesis of human asthma, individuals with mild asthma were exposed to allergen. The expression of ICOS on T cells present in the bronchoalveolar lavage (BAL) fluid collected 42 hours after segmental allergen provocation was analyzed by flow cytometry, as described in Exhibit 6. These data, described in Exhibit 7, were correlated with immunohistological studies on ICOS expression in bronchial biopsies taken at the time the performed BAL was performed.

13. The data clearly demonstrate that a very substantial proportion of the infiltrating T cells found in the submucosa and in the epithelium bear significant levels of ICOS. These ICOS-positive T cells transmigrate into the bronchial space on allergen exposure.

14. The finding that ICOS is involved in human lung inflammation validates the results obtained by Gonzalo demonstrating the inhibition of hallmarks of asthma by ICOS inhibitory molecules in a mouse model of asthma, and signifies that ICOS inhibitory compounds will have similar effects on human asthma. Thus, taken together, the human data presented herein and the mouse model data shown by Gonzalo strongly indicate that: a) ICOS participates in the pathogenesis of allergic asthma in humans; and b) amelioration of asthma symptoms in humans can be achieved by administration of antibodies that recognize the ICOS polypeptide or by administration of ICOS polypeptides.

15. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: October 4, 2001

Richard Kroczek

Attachments:

Exhibit 1: *Curriculum Vitae* of Richard Kroczek

Exhibit 2: Gonzalo *et al.*, 2001, "ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses," Nat. Immunol. 2(7):597-604.

Exhibit 3: Wills-Karp, 2000, Immunopharmacology 48:263-268.

Exhibit 4: Keane-Myers *et al.*, 1998, "Development of murine allergic asthma is dependent upon B7-2 costimulation," J. Immunol. 160:1036-1043.

Exhibit 5: Mathur *et al.*, 1999, "CD28 interaction with either CD80 or CD86 are sufficient to induce allergic airway inflammation in mice," Am. J. Respir. Cell. Mol. Biol. 21:498-509.

Exhibit 6: Materials and Methods for Human Asthma Analysis

Exhibit 7: Results for Human Asthma Analysis

CURRICULUM VITAE

Name: Richard Kroczek, M.D.

Occupation: Professor, Molecular Immunology
Robert Koch-Institute
Nordufer 20, 13353 Berlin, Germany

Phone: (+49) 30 4547-2450
Fax: (+49) 30 4547-2603
e-mail: kroczek@rki.de

Personal Information: born November 3, 1952 in Orlau
Nationality: German

Education and Training:

1964 - 1973	Attending the Hans-Leinberger-Gymnasium in Landshut, Germany; graduated first in the class of 1973
1973 - 1976	Pre-clinical studies at the University of Kiel
1976 - 1977	Medical School, University of Bonn
1977 - 1978	Westminster Hospital Medical School, London, supported by a grant from the Deutscher Akademischer Austauschdienst
1978 - 1981	Continuation of clinical studies at the University of Bonn
1981	Final Medical Exam ("Staatsexamen"); Doctoral thesis; Medical License
1981 - 1983	Residency in Pediatrics at the Munich University Children's Hospital
1983	American Medical Exam (VQE)
1984 - 1986	Postdoctoral Fellow in Immunology with Dr. Ethan Shevach in the Laboratory of Allergy and Infectious Diseases, NIH, USA. Supported by a grant from the Deutsche Forschungsgemeinschaft. Research topics: Role of Thy-1 in T-cell activation, action of cyclosporin A
1986	Research fellow of the Fogarty Foundation

1986 - 1987	Postdoctoral fellow at the Max-Planck-Institute for Immunobiology in Freiburg

Employment:

1987 - 1992	Head of a research group at the Max-Planck-Society Research Unit for Immunology in Erlangen, Germany
1990	Habilitation at the University of Erlangen; faculty member of the university
1997	Professor, University of Erlangen
1993 -	Head, Molecular Immunology, Robert Koch-Institute, Berlin
1999	Offered chair in immunology at the Free University of Berlin (not accepted)

Current research: Molecular mechanisms of early T cell activation, T cell/B cell cooperation, T cell/monocyte cooperation, T cell/dendritic cell cooperation focus on the function of CD40 Ligand, ATAC and ICOS molecules in vitro and in vivo

Professional and scientific activities:

Member of the German Society for Immunology.

Reviewer for various scientific journals (European Journal of Immunology, Journal of Immunology, European Journal of Biochemistry, Blood, Journal of Clinical Investigation, Nature Medicine).

Reviewer for various scientific societies and funding agencies.

Honors:

Science prize of the SmithKline Beecham Foundation 1999.

PUBLICATIONS

1. Kroczek RA, Däumling S, Belohradsky BH.
Kawasaki-Syndrom. 4 Todesfälle in der Bundesrepublik. Diagnose, Therapie und Prognose der kardialen Komplikationen.
Pädiatrische Praxis 28 (1983) 491-495

2. Mezger J, Kroczek RA, Belohradsky BH, Remberger K.
Das Kawasaki-Syndrom.
Medizinische Welt 34 (1983) 1085-1090

3. Kroczek RA.
Congenital chyloperitoneum: Direct comparison of medium-chain triglyceride treatment with total parenteral nutrition.
European Journal of Pediatrics 144 (1985) 77-79

4. Kroczek RA, Mühlbauer W, Zimmermann S.
Cloverleaf skull associated with Pfeiffer-syndrome: pathology
and management.
European Journal of Pediatrics 145 (1986) 442-445

5. Gunter KC, Kroczek RA, Shevach EM, Germain RN.
Functional expression of the murine Thy-1.2 gene in transfected human T cells.
Journal of Experimental Medicine 163 (1986) 285-300

6. Kroczek RA, Gunter KC, Seligmann B, Shevach EM.
Induction of T cell activation by monoclonal anti-Thy-1 antibodies.
Journal of Immunology 136 (1986) 4379-4384

7. Kroczek RA, Gunter KC, Germain RN, Shevach EM.
Thy-1 functions as a signal transduction molecule in T-lymphocytes and transfected B-lymphocytes.
Nature 322 (1986) 181-184

8. Malek TR, Ortega G, Chan C, Kroczek RA, Shevach EM.
Role of Ly-6 in lymphocyte activation. II. Induction of T cell activation by monoclonal anti-Ly-6 antibodies.
Journal of Experimental Medicine 164 (1986) 709-722

9. Gunter KC, Germain RN, Kroczek RA, Saito T, Yokoyama WM, Chan C, Weiss A, Shevach EM.
Thy-1-mediated T-cell activation requires co-expression of CD3/Ti complex.
Nature 326 (1987) 505-507

10. Kroczek RA, Black CDV, Barbet J, Edison LJ, Shevach EM.
Induction of IL-2 receptor expression in vivo: Response to allogeneic cells.
Transplantation 44 (1987) 547-553

11. Kroczek RA, Black CDV, Barbet J, Shevach EM.
Mechanism of action of Cyclosporin A in vivo. Cyclosporin A fails to inhibit T lymphocyte activation in response to alloantigens.
Journal of Immunology 139 (1987) 3597-3603

12. Black CDV, Kroczek RA, Barbet J, Weinstein J, Shevach EM.
Induction of IL-2 receptor expression in vivo: Response to Concanavalin A.
Cellular Immunology 111 (1988) 420-432

13. Kroczek RA.
Immediate visualization of blotted RNA in Northern analysis.
Nucleic Acids Research 17 (1989) 9497

14. Kroczek RA, Siebert E.
Optimization of Northern analysis by vacuum-blotting, RNA-transfer visualization and UV-fixation.
Analytical Biochemistry 184 (1990) 90-95

15. Potocnik AJ, Kinne R, Menninger H, Zacher J, Emmrich F, Kroczek RA.
Expression of activation antigens on T cells in rheumatoid arthritis patients.
Scandinavian Journal of Immunology 31 (1990) 213-224

16. Potocnik AJ, Menninger H, Yang SY, Pirner K, Krause, A, Burmester GR, Bröker BM, Hept P, Weseloh G, Michels H, Truckenbrodt H, Emmrich F, Kroczek RA.
Expression of the CD2 activation epitope T11-3 (CD2R) on T-cells in rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, and lyme disease: phenotypic and functional analysis.
Scandinavian Journal of Immunology 34 (1991) 351-358

17. Korthäuer U, Hennerkes B, Menninger H, Mages HW, Zacher J, Potocnik AJ, Kroczek RA.
Oligoclonal T-cells in rheumatoid arthritis: identification strategy and molecular characterization of a clonal T-cell receptor.
Scandinavian Journal of Immunology 36 (1992) 855-863

18. Graf D, Korthäuer U, Mages HW, Senger G, Kroczek RA.
Cloning of TRAP, a ligand for CD40 on human T cells.
European Journal of Immunology 22 (1992) 3191-3194

19. Mages HW, Stamminger T, Rilke O, Bravo R, Kroczek RA.
Expression of PILOT, a putative transcription factor, requires in T-cells two signals and is cyclosporin A sensitive.
International Immunology 5 (1993) 63-67

20. Korthäuer U, Graf D, Mages HW, Brière F, Padayachee M, Malcolm S, Ugazio AG, Notarangelo LD, Levinsky RJ, Kroczek RA.
Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM.
Nature 361 (1993) 539-541

21. Bröker BM, Korthäuer U, Heppt P, Weseloh G, de la Camp R, Kroczek RA, Emmrich F.
Biased T cell receptor V gene usage in rheumatoid arthritis. Oligoclonal expansion of T cells expressing Vß 2 genes in synovial fluid but not in peripheral blood.
Arthritis and Rheumatism 36 (1993) 1234-1243

22. Zimmermann S, Becker-Perez I, Beuscher HU, Kroczek RA, Roellinghoff M, Solbach W.
Leishmania major parasites share an epitope with the murine CD3-T cell receptor complex.
European Journal of Immunology 24 (1994) 503-507

23. Villa A, Notarangelo LD, DiSanto JP, Macchi PP, Strina D, Frattini A, Lucchini F, Patrosso CM, Giliani S, Mantuano E, Agosti S, Nocera G, Kroczek RA, Fischer A, Ugazio AG, de Saint Basile G, Vezzoni P.
Organization of the human CD40L gene - implications for molecular defects in X-chromosome-linked hyper-IgM syndrome and prenatal diagnosis.
Proceedings of the National Academy of Sciences 91 (1994) 2110-2114

24. Brugnoni D, Airo P, Graf D, Marconi M, Lebowitz M, Plebani A, Giliani S, Malacarne F, Cattaneo R, Ugazio AG, Albertini A, Kroczek RA, Notarangelo LD.
Ineffective expression of CD40 ligand on cord blood T cells may contribute to poor immunoglobulin productio in the newborn.
European Journal of Immunology 24 (1994) 1919-1924

25. Callard RE, Smith SH, Herbert J, Morgan G, Padayachee M, Lederman S, Chess L, Kroczek RA, Fanslow WC, Armitage RJ.
CD40 ligand (CD40L) expression and B cell function in agammaglobulinemia with normal or elevated levels of IgM (HIM). Comparison of X-linked, autosomal recessive and non-X linked forms of the disease, and obligate carriers.
Journal of Immunology 153 (1994) 3295-3306

26. Mages HW, Rilke O, Bravo R, Senger G, Kroczek RA.
NOT, a human immediate-early response gene closely related to the steroid/thyroid hormone receptor NAK1/TR3.
Molecular Endocrinology 8 (1994) 1583-1591

27. Durandy A, de Saint Basile G, Lisowska-Grospierre B, Gauchat JF, Forveille M, Kroczek RA, Bonnefoy JY, Fischer A.
Undetectable CD40 ligand expression on T cells and low B cell responses to CD40 binding agonists in human newborns.
Journal of Immunology 154 (1995) 1560-1568

28. Kinne RW, Boehm S, Iftner T, Aigner T, Vornehm S, Weseloh G, Bravo R, Emmrich F, Kroczek RA.
Synovial fibroblast-like cells strongly express Jun-B and C-Fos proto-oncogenes in rheumatoid- and osteoarthritis.
Scandinavian Journal of Rheumatology S 101 (1995) 121-125

29. Müller S, Dorner B, Korthäuer U, Mages HW, D'Apuzzo M, Senger G, Kroczek RA.
Cloning of ATAC, an activation-induced, chemokine-related molecule exclusively expressed in $CD8^+$ T lymphocytes.
European Journal of Immunology 25 (1995) 1744-1748

30. Graf D, Müller S, Korthäuer U, van Kooten C, Weise C, Kroczek RA.
A soluble form of TRAP (CD40 ligand) is rapidly released after T cell activation.
European Journal of Immunology 25 (1995) 1749-1754

31. Ludewig B, Graf D, Gelderblom HR, Becker Y, Kroczek RA, Pauli G.
Spontaneous apoptosis of dendritic cells is efficiently inhibited by TRAP (CD40-ligand) and TNF-α, but strongly enhanced by interleukin-10.
European Journal of Immunology 25 (1995) 1943-1950

32. Ruggiero G, Caceres EM, Voordouw A, Noteboom E, Graf D, Kroczek RA, Spits H.
CD40 expressed on thymic epithelial cells provides costimulation for proliferation but not for apoptosis of human thymocytes.
Journal of Immunology 156 (1996) 3737-3746

33. Brugnoni D, Airo P, Graf D, Marconi M, Molinari C, Braga D, Malacarne F, Soresina A, Ugazio AG, Cattaneo R, Kroczek RA, Notarangelo LD.
Ontogeny of CD40 ligand expression by activated peripheral blood lymphocytes in humans.
Immunology Letters 49 (1996) 27-30

34. Nasert S, Burtchen N, Kussebi F, Millner M, Kroczek R, Jung T, Schwinzer R, Wahn U, Renz H.
Stimulation of IgE and IgA production by CD45 RA T helper cells in atopic patients
Journal of Immunology 157 (1996) 441-448

35. Feske S, Müller JM, Graf D, Kroczek RA, Dräger R, Niemeyer C, Baeuerle PA, Peter HH, Schlesier M.
Severe combined immunodeficiency due to defective binding of the nuclear factor of activated T cells in T lymphocytes of two male siblings.
European Journal of Immunology 26 (1996) 2119-2126

36. Ludewig B, Henn V, Schröder, J.M., Graf D, Kroczek RA.
Induction, regulation, and function of soluble TRAP (CD40 ligand) during interaction of primary $CD4^+CD45RA^+$ T cells with dendritic cells.
European Journal of Immunology 26 (1996) 3137-3143

38. Schultz A, Greiner A, Nenninger R, Schoemig D, Wilisch A, Oswald E, Kroczek RA, Schalkef B, Mueller-Hermelink HK, Marx A.
CD40 als Vermittler von Proliferation in normalem und neoplastischem Thymusepithel.
Verhandlungen der Deutschen Gesellschaft für Pathologie 80 (1996) 250-255

39. Dorner B, Müller S, Entschladen F, Schröder JM, Franke P, Kraft R, Friedl P, Clark-Lewis I, Kroczek RA.
Purification, structural analysis and function of natural ATAC, a cytokine expressed in $CD8^+$ T cells.
Journal of Biological Chemistry, 272 (1997) 8817-8823

40. Hermes B, Worm M, Nowak F, Kroczek RA, Stein H, Henz BM.
Upregulation of CD40 and CD40-ligand expression in IgE-associated cutaneous diseases.
Acta Dermatologica Veneorologica 77 (1997) 441-445

41. Henn V, Slupsky JR, Gräfe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, Kroczek RA.
CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells.
Nature 391 (1998) 591-594

42. Slupsky JR, Kalbas M, Willuweit A, Henn V, Kroczek RA, Müller-Berghaus G.
Activated platelets induce tissue factor expression on human umbilical vein endothelial cells by ligation of CD40.
Thrombosis and Haemostasis 80 (1998) 1008-1014

43. Mages HW, Baag R, Steiner B, Kroczek RA.
Utilization of an NF-ATp binding promoter element for EGR3 expression in T cells but not fibroblasts provides a molecular model for the lymphoid cell-specific effect of Cyclosporin A.
Molecular and Cellular Biology 18 (1998) 7157-7165

44. Greiner A, Knoerr C, Qin Y, Schultz A, Marx A, Kroczek RA, Mueller-Hermelink HK
CD40 ligand and autoantigen are involved in the pathogenesis of low-grade B-cell lymphomas of mucosa-associated lymphoid tissue.
Developmental Immunology, 6 (1998) 187-195

45. Hutloff A, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R, Anagnostopoulos I, Kroczek RA.
ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28.
Nature 397 (1999) 263-266

46. Mages HW, Hutloff A, Heuck C, Büchner K, Himmelbauer H, Oliveri F, Kroczek RA.
Molecular cloning and characterization of murine ICOS and identification of B7h as ICOS ligand.
European Journal of Immunology 30 (2000) 1040-1047

47. Rengarajan J, Mittelstadt PR, Mages HW, Gerth AJ, Kroczek RA, Ashwell JD, Glimcher LH.
Sequential involvement of NFAT and Egr transcription factors in FasL regulation.
Immunity 12 (2000) 293-300

48. Lienenlüke B, Germann T, Kroczek RA, Hecker M.
CD154 stimulation of interleukin-12 synthesis in human endothelial cells.
European Journal of Immunology 30 (2000) 2864-2870

49. Beier KC, Hutloff A, Dittrich AM, Heuck C, Rauch A, Büchner K, Ludewig B, Ochs HD, Mages HW, Kroczek RA.
Induction, binding specificity and function of human ICOS
European Journal of Immunology 30 (2000) 3707-3717

50. Ebner S, Ratzinger G, Krösbacher B, Schmuth M, Weiss A, Reider D, Kroczek RA, Herold M, Heufler C, Fritsch P, Romani N.
Production of IL-12 by human monocyte-derived dendritic cells is optimal when the stimulus is given at the onset of maturation, and is further enhanced by IL-4.
Journal of Immunology 166 (2001) 633-641

51. Henn V, Steinbach S, Büchner K, Presek P, Kroczek RA.
The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporarily limited by co-expressed CD40.
Blood 98 (2001) 1047-1054

52. Gonzalo JA, Tian J, Delaney T, Corcoran J, Rottman JB, Lora J, Al-garawi A, Kroczek R, Gutierrez-Ramos JC, Coyle AJ. ICOS is critical for T helper cell-mediated lung mucosal inflammatory responses.
Nature Immunology 2 (2001) 597-604